Effect of Guanine Nucleotide Exchange Factor Inhibitors on pU$_L$34 Localization in Cells Infected with Herpes Simplex Virus Type-1

R. Scott Dudis; Joel D. Baines

Cornell College of Veterinary Medicine, Department of Microbiology and Immunology, Cornell University

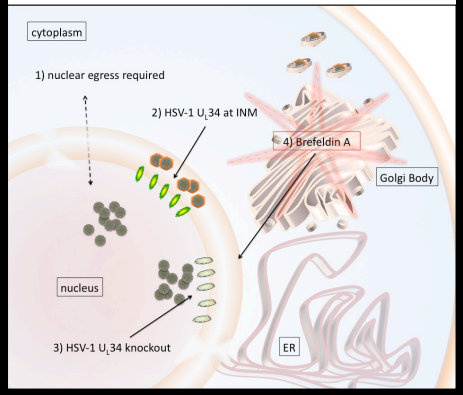
Abstract

Herpes simplex virus type-1 (HSV-1) buds from the nucleus into the inner nuclear membrane (INM), where it forms enveloped capsids in the perinuclear space. Viral protein U$_L$34 localizes at the INM, where it recruits and co-localizes other viral and cellular proteins and is required for viral budding at the INM. Brefeldin A (BFA) blocks viral egress through an unknown mechanism. It is known that BFA inhibits ADP ribosylation factor (ARF) family proteins, which suggests that these proteins may be involved in viral egress. To explore this function, we treated HSV-infected cells with BFA and observed pU$_L$34 localization in the nucleus by immunofluorescence and confocal microscopy. Preliminary results show that infected cells treated with BFA or Tyrphostin AG 1478 display a mislocalization of pU$_L$34 at the INM uncharacteristic of the smooth distribution of the protein in infected cells that were not treated with BFA. Future aspects of this project will focus on specific targets of BFA, such as Arf1p. This study is relevant because there are U$_L$34 homologues in all herpesvirus family members and there are no known homologues in cellular genes. It is, therefore, an obvious potential target for pharmaceutical intervention.

Background

Herpesvirus:
The herpesviridae family includes numerous viruses that infect both animals and humans. There are many common features across the members. They all have dsDNA (approximately 100 Mda) with an RNA transcription step in the nucleus. They share an icosahedral capsid structure and a lipid bilyaer envelope. Clinical features in humans are typically oral ulcers or vesicular lesions of specific epithelial cells and nerual ganglion. Infection persists in latent and lytic periods after primary infection clears.

HSV-1 Egress Pathway:
HSV viral protein U$_L$34 is required for HSV egress from the nucleus (all sequenced herpesviruses have a U$_L$34 gene homologue). pU$_L$34 is made in the ER and then recruited to the inner nuclear membrane (INM). It co-localizes with other viral and cellular proteins related to envelopment. Enveloped capsids bud through the INM into the perinuclear space. INM enclosed capsids fuse with the ONM for de-envelopment. Virion then moves from ER to Golgi network and exocytoses from the cell.



Hypothesis

The U$_L$34 protein of HSV-1 requires ADP-ribosylation factor (Arf) proteins for proper viral egress from the nucleus.

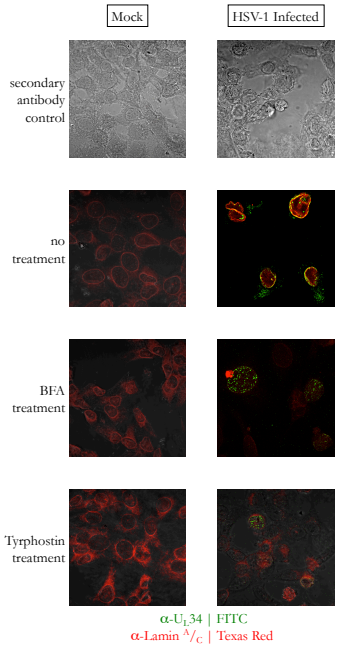
Data & Results

Guanine Nucleotide Exchange Factor Inhibitors

Brefeldin A:
- fungal lactone antibiotic
- known effects: inhibits protein transport from ER to Golgi
- known effects: induces retrograde transport from Golgi to ER
- known effects: blocks nuclear egress of HSV-1
- BFA targets a GTPase called Arf1p (also known as a guanine nucleotide exchange factor protein)
- Arf1p is involved in recruitment of coat proteins to intracellular membranes for vesicular transport

Tyrphostin AG 1478:
- abbreviation of tyrosine phosphorylation inhibitor (a.k.a. TKIs)
- synthetic ligand of the epidermal growth factor receptor family
- more discriminating in inhibition process than BFA (higher specificity of study)

Figures:
- Experimental treatment is presented adjacent to controls for each sample.
- Confocal microscopy settings were adjusted to eliminate non-specific background (based on controls using only 2° antibody).



Conclusions

HEp-2 cells infected with HSV-1 and treated with BFA or Tyrphostin AG 1478 display an accumulation of U$_L$34 in a punctate pattern throughout the nucleoplasm. Untreated infected cells show a smooth distribution of U$_L$34 around the nuclear membrane.

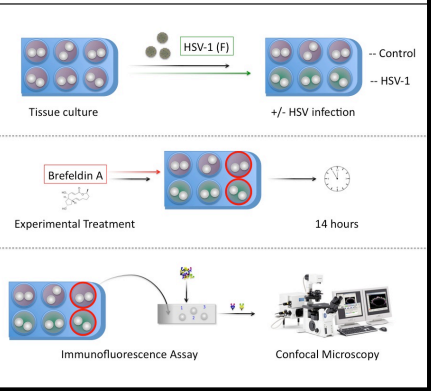
BFA and Tyrphostin AG 1478 cause a mislocalization of HSV-1 pU$_L$34 in cells infected with HSV-1. Further study is warranted.

Acknowledgments

Special thanks go to the members of the Baines laboratory: Joel Baines, Liz Wills, Serena Chang, Kari Roberts, and Kui Yang, for their guidance and encouragement. This project was part of the Cornell Veterinary Investigator Program, at the Cornell College of Veterinary Medicine, which is led by Robert Gilmour, Janna Lamey, and Casey Isham. Funding was provided through the NIH Kirschstein-NRSA Training Program grants.

Materials & Methods

Viral stock of HSV-1(F) strain was grown in Vero cells. HEp-2 cells were plated at 75% confluency on glass coverslips in 6-well plates with 2 coverslips per well. Cells were infected at an multiplicity of infection (moi) of 10 and incubated for 14 hours. Brefeldin and Tyrphostin treatment was performed 3 hours post-infection (hpi) at 5μM and 14μM, respectively. Cell were fixed in -20ºC methanol. Autofluorescence was quenched with 50 mM NH_4Cl. Two blocking steps were performed (10% human/10% goat serum in PBS, 10% BlokHen in PBS). FITC green marker (1:400 anti-chicken) was tagged to α-U$_L$34. Texas Red red marker (1:100 anti-mouse) was tagged to α-Lamin $^A/_C$. Fluorescence was visualized with an Olympus IX70 confocal microscope.



Current & Future Work

To asses the role of specific ARF family protein members (there are currently six known ARFs), siRNA targeted knock-down of individual genes is being investigated, beginning with Arf1p. This gene knock-down will be verified by immunoblot and then effects visualized by immunofluorescence.

Additionally, observations of the Golgi in the presence of BFA and Tyrphostin will be made to mesaure their effect on the cell and validate previous studies.

Finally, the role of other viral and host proteins, such as U$_L$31 and U$_S$3, will be included with the current technique and experimental design.

References

- Dasgupta, Anindya, and Wilson, Duncan. Evaluation of the Primary Effect of Brefeldin A Treatment Upon Herpes Simplex Virus Assembly. Journal of Virology, July 2001; 82: 1561 – 1567.
- Richard J. Roller. Nuclear Egress of Herpesviruses. Virologica Sinica, December, 2008; Volume 23, Number 6: 406 – 415
- Reynolds, Ashley, et al. UL31 And UL31 Proteins of Herpes Simplex Virust Type 1 Form a Complex that Accumulates at the Nuclear Rim and Is Required for Envelopment of Nucleocapsids. Journal of Virology, September 2001; Volume 75, Number 18: 8803 - 8817